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Lack of Predictive Value for Progression of Dissociated Circulating P24 Antigen in Human Immunodeficiency Virus Type 1-Infected Black Patients

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In human immunodeficiency virus type 1 (HIV)-1-infected Black people, the circulating p24 antigen is hidden frequently in immune complexes, because of high titers of serum anti-p24 antibodies. In order to evaluate the prognostic values for progression of free and dissociated serum p24 antigen in Black people, sera from 45 HIV-1-infected Black patients, all at non-AIDS stages, were evaluated prospectively for p24 antigen by several assays: circulating free p24 antigen was measured by immunocapture ELISA only (method 1) and with ELAST™ amplification (method 2), and dissociated p24 antigen determined after glycine-HCl pretreatment of serum, by immunocapture ELISA only (method 3) and with ELAST™ amplification (method 4). Serum CD4 and CD8 cell counts, β_2-microglobulin, and total IgA were determined also at least twice a year. Clinical events for AIDS were those included in the 1986 CDC classification for HIV infection. At entry, p24 antigen was found in 3 (6.7%) patients by method 1, in 7 (15.6%) by method 2, in 14 (31.1%) by method 3, and in 22 (48.9%) by method 4. Methods 3 and 4 were more sensitive than method 1 ($P < 0.001$) and method 2 ($P < 0.001$). The mean follow-up was 30 months. The free symptom survival times (mean ± SD months) were significantly lower in patients being p24 antigen positive by method 1 [(+) 33 ± 27 vs. (−) 61 ± 15, $P = 0.03$], but they were similar in patients positive and in those negative for p24 antigen determined by method 2 [(+) 71 ± 17 vs. (−) 74 ± 9, $P = 0.54$], method 3 [(+) 76 ± 12 vs. (−) 69 ± 13.2, $P = 0.80$], and method 4 [(+) 79 ± 9 vs. (−) 63 ± 7, $P = 0.71$]. At 24 months, p24 antigen positivity did not correlate either with CD4 or CD4/CD8 slops, nor with β_2-microglobulin or IgA variations. By contrast, a CD4 cell count below 200/mm^3 at entry was significantly associated with disease progression. In conclusion, dissociated p24 antigenemia does not appear as a useful surrogate marker for progression in HIV-1-infected Black people.

KEY WORDS: p24 antigen, immune complex dissociation, ELAST™ amplification procedure, surrogate marker, HIV-1 infection, Black people

INTRODUCTION

Several biological surrogate markers have been suggested for estimating the progression of patients infected with human immunodeficiency virus type 1 (HIV-1), including circulating p24 antigen [Moss et al., 1988; Harry et al., 1989; Fahey et al., 1990]. One potential limitation of p24 antigenemia as a prognostic monitoring marker is the low prevalence of detectable free p24 antigenemia in defined populations. For example, in asymptomatic HIV-infected individuals from Western countries, p24 antigenemia ranges from 3 to 10%, while in AIDS patients, its prevalence is more than 60% [Harry et al., 1989]. Racial heterogeneity for p24 antigenemia has also been emphasized. Indeed, in Black people from the United States [Chaisson et al., 1991; Brown et al., 1991] and Africa [Baillou et al., 1987; Katzenstein et al., 1990; Kaleebu et al., 1991; Brown et al., 1991; Ayehunie et al., 1992; Bélec et al., 1993], a lower serum free p24 antigen prevalence than in Caucasians has been documented at asymptomatic to advanced stages of HIV-1 infection.

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Acid pretreatment of the serum has been recognized to increase significantly the sensitivity of p24 antigen detection [Nishanian et al., 1990; Kestens et al., 1991; Pokriefla et al., 1993; Lillo et al., 1993; Brown et al., 1991], especially in the early stages of HIV infection [Nishanian et al., 1990]; this has also been noted in HIV-1-infected Black individuals [Kestens et al., 1991; Bélec et al., 1993; Brown et al., 1995]. Furthermore, dissociated p24 antigen has been recognized to be more efficient as a marker of disease progression than free p24 antigen [Bollinger et al., 1992; Morand-Joubert et al., 1994]. We therefore evaluated the prognostic value of progression of dissociated p24 antigen in HIV-1-infected Black patients at non-AIDS stages.

MATERIALS AND METHODS

Patients

HIV-1-infected Black adults from three different hospitals around Paris (Hôpital Villeneuve St Georges, Hôpital Gonesse, and Hôpital Meaux) were prospectively and consecutively followed. All patients included in this study fulfilled the criteria of stages II, III, IV_A, or IV_{C2}, according to the 1986 Centers for Disease Control (CDC) classification for HIV infection, and were followed-up during at least 12 months, with three visits per year. Thirty-one patients received antiretroviral therapy (zidovudine or didanosine) at entry or during follow-up.

Serum samples were obtained from each patient and kept frozen at −30°C until processing. Clinical and routine laboratory data [CD4 and CD8 T cells counts, β_2-microglobulin, serum total IgA and IgG] were collected from each patient file using a standardized questionnaire.

Detection and Quantification of Free and Dissociated p24 Antigen

Circulating free p24 antigen was detected and quantified using immunocapture ELISA (HIV-p24 Core Profile ELISA, Du Pont de Nemours, Boston, MA), without (method 1) and with ELAST™ (NEP-116 ELAST™ ELISA Amplification system, Du Pont de Nemours) amplification (method 2), according to the manufacturer's instructions. The thresholds of positivity corresponded to the mean of three negative controls plus 0.08 OD units by immunocapture ELISA, and to the mean of three negative controls (amplified by ELAST™) plus 0.06 OD units by immunocapture ELISA plus ELAST™ amplification. The concentration of p24 antigen was estimated from a standard curve of known concentrations of recombinant p24 antigen, treated or not by ELAST™ amplification.

Circulating dissociated p24 antigen concentration was evaluated by immunocapture ELISA (Du Pont de Nemours) after dissociation of immune complexes with HCl-glycine buffer (Du Pont de Nemours), without (method 3) and with ELAST™ amplification (method 4), according to the manufacturer's recommendations. Briefly, the acid dissociated procedure was carried out as follow. One hundred microliters of serum was mixed with 100 µl of 1.5 M glycine (pH 2) for 60 min at 37°C, and then neutralized with 100 µl of 3.5 M Tris buffer (pH 9) for 10 min at room temperature. Two hundred microliters of the mixture was taken for the p24 antigen assay. The concentration of p24 antigen was estimated from a standard curve of known concentrations of recombinant p24 antigen that had been treated similarly with glycine-HCl. The thresholds of positivity were the mean of three negative controls (treated by glycine-HCl) plus 0.08 OD units by immunocapture ELISA, and the mean of three negative controls (treated by glycine-HCl and amplified by ELAST™) plus 0.06 OD units by immunocapture ELISA plus ELAST™ amplification. The ranges of the standard curve were 0 to 100 pg/ml for methods 1 and 3 and 0 to 16 pg/ml for methods 2 and 4.

All positive samples were confirmed by a neutralization assay using HIV p24 antigen blocking antibody (Du Pont de Nemours). The serum, treated eventually by the ELAST™ amplification or by the immune complexes dissociation procedure, was considered confirmed as positive for p24 antigen if a 50% or greater decrease in p24 antigen reactivity was observed after incubation with an excess of anti-p24 antibody.

Judgment Criteria and Statistical Analysis

In order to establish the predictive value of laboratory markers, a clinical parameter of disease progression, named as an event, was defined as one of the diseases included in the 1986 CDC classification for HIV infection. This event illustrates the progression from one stage to another (i.e., from CDC stage II to CDC stage IV_{C2} or from CDC stage IV_{C2} to CDC stage IV_{C1}), or as an AIDS-related death. In the subgroup of patients whose CD4 T-cell count at entry was above 200/mm^3, progressors were defined as those with a CD4 T-cell count below 200/m^3 at the end of the follow up and nonprogressors as those with CD4 T-cell counts still above 200/mm^3 at the same time.

Kaplan-Meier plots were used to illustrate the distributions of event-free time for different groups of patients according to the results of p24 antigenemia by each method and according to the level of surrogate markers at entry. The Mantel Cox log rank test was used to compare each subgroup and to illustrate the statistics. Kaplan-Meier plots and statistics were calculated using BMDP Statistical Software. Predictive value of circulating p24 antigen on surrogate marker variation was estimated using the nonparametric Kruskall-Wallis and Wilcoxon tests.

RESULTS

Study Population

Forty-five HIV-1-infected Black patients were included, their median ± SD age was 34 ± 7.5 years. The geographical origin was sub-Saharan Africa for 34, Haiti for 8, and Sri Lanka for 3. At inclusion, 34 patients were classified at CDC stage II and 11 at CDC stage IV_{C2}. Eight patients were females and 37 were males. Mean follow-up was 30 months; one patient was followed up during 12 months and 8 patients more than 48 months.

TABLE I. Median, Mean, and Standard Deviation of CD4+ T Cells, CD8+ T cells, β_2-Microglobulinemia (mg/l), IgA (g/l), and IgG (g/l) and mean CD4+ and CD8+ T-Cell Percentage Among the 45 HIV-1-Infected Black Patients

	Overall population (N = 45)	Stage II (N = 34)	Stage IV C2 (N = 11)
CD4+ T cells			
Median	262	330	87
Mean (SD)	300 (200)	361 (219)	113 (91)
CD4 (%)	20	20	10
CD8+ T cells			
Median	893	879	893
Mean (SD)	904 (441)	914 (481)	876 (314)
CD8 (%)	58	51	59
β_2-microglobulinemia (mg/l)			
Median	2.21	2.04	3.11
Mean (SD)	2.32 (0.91)	2.11 (0.82)	2.99 (0.87)
IgA			
Median	2.22	2.12	3.19
Mean (SD)	2.82 (1.81)	2.36 (0.93)	4.15 (2.92)
IgG			
Median	22.7	21.30	23.9
Mean (SD)	27.1 (12.8)	27.1 (12.9)	27.0 (13.4)

TABLE II. Free and Dissociated p24 Antigenemia Detection According to CDC Stage of HIV Disease

	Stage II	Stage IV C2	*P*
Free p24 antigenemia (pg/ml)			
ELISA Method 1	1/34	2/11	0.08
ELISA + ELAST Method 2	5/34	2/11	0.78
Dissociated p24 antigenemia (pg/ml)			
ELISA Method 3	9/34	5/11	0.24
ELISA + ELAST Method 4	14/34	8/11	0.06

Cross-Sectional Study

At entry, CD4 T-cell counts were significantly lower in patients at CDC stage IV_{C2} than in patients at CDC stage II ($P < 0.01$). β_2-microglobulin and IgA serum levels were significantly higher (respectively, $P < 0.02$ and $P < 0.03$) (Table I). CD8 T-cell counts and IgG serum levels were similar whatever the disease stages.

Results for free and dissociated p24 antigenemia detection and quantification by the four different methods are presented in Tables II and III according to the stages of the disease. By method 1, circulating free p24 antigen was detected in only three (6.7%) patients. By method 2, the number of free p24 antigen-positive sera was detected in seven (15.6%) patients, but this was not significant ($P = 0.17$). All positive sera by Methods 1 and 2 were confirmed by neutralization.

By method 3, the number of neutralized, dissociated p24 antigen-positive sera was increased significantly to 14 (31.1%) patients by comparison with method 1 ($P = 0.003$), but not with method 2 ($P = 0.08$). By method 4, the number of neutralized, dissociated p24 antigen-positive sera increased to 22 (48.8%) patients. Method 4 was more sensitive than methods 1 ($P = 0.0001$) and 2 ($P = 0.001$), but not more than method 3 ($P = 0.08$).

According to the stage of HIV infection, the number of p24 antigen-positive sera by method 4 was slightly higher in patients at CDC stage IV_{C2} than in those at CDC stage II ($P = 0.06$); no difference was observed with other methods. The concentrations of p24 antigenemia (median, range, and mean ± standard error in pg/ml) did not differ significantly according to the stages of the disease (Table III).

LONGITUDINAL STUDY

The relations between the progression to symptomatic events or to AIDS according to the various studied biological markers at study enrollment are illustrated in Kaplan-Meier plots of the frequency of occurrence of such events.

In a first approach, survival curves were drawn for the classical surrogate markers (e.g., CD4 T cells, serum β_2-microglobulin, and IgA). For each marker, the patients were divided into two groups according to the values at entry by comparison with a cutoff value: 200/mm^3 for CD4 T cells, 2.5 mg/l for serum β_2-microglobulin, and 4 g/l for serum IgA. A strong association between CD4 T-cell count and subsequent disease progression was observed (Fig. 1A): 70.6% (SD = 20.8) of patients with more than 200 CD4 T-cell count per mm^3 at enrollment remained free of event after a 24-month follow-up, compared with only 30.6% (SD = 16.4) of patients having a CD4 T-cell count below 200/mm^3 at entry (Mantel Cox = 8.68, $P = 0.003$). For serum β_2-microglobulin, the probability to remain free of event in patients exhibiting a level higher than 2.5 mg/l at entry was 41.8% (SD = 20.4) after 24 months (Fig. 1B); it did not differ from the 64.0% (SD = 16.7) survival rate observed in patients exhibiting a level below 2.5 mg/l (Mantel Cox = 1.78, $P = 0.18$). Lastly, for serum IgA, the probability to remain free of event in patients exhibiting a level higher than 4 g/l at entry, 60.1% (SD = 21.9) after 24 months, was similar to the survival rate found in

TABLE III. Median, Range, Mean, and Standard Error (SE) of p24 Antigenemia According to the Method of Determination and CDC Stage of HIV Disease

	Overall population (N = 45)		Stage II (N = 34)		Stage IV C2 (N = 11)		Kruskall Wallis and P value*
Free p24 antigenemia (pg/ml)							
ELISA	0	(0–70)	0	(0–8)	0	(0–70)	3.03
Method 1	1.9	(SE = 1.6)	0.24	(SE = 0.23)	6.95	(SE = 6.3)	$P = 0.08$
ELISA + ELAST	0	(0–47)	0	(0–29)	0	(0–47)	0.13
Method 2	2.3	(SE = 1.2)	1.5	(SE = 0.89)	4.86	(SE = 4.2)	$P = 0.72$
Dissociated p24 antigenemia (pg/ml)							
ELISA	0	(0–234)	0	(0–218)	0	(0–234)	1.13
Method 3	21	(SE = 7.7)	17	(SE = 7.9)	32.3	(SE = 20.8)	$P = 0.28$
ELISA + ELAST	0	(0–20)	0	(0–20)	0	(0–20)	3.2
Method 4	5.4	(SE = 1.2)	4.6	(SE = 1.3)	7.91	(SE = 2.5)	$P = 0.07$

*P values of nonparametric means comparison according to the CDC stage for each method of p24 antigenemia determination are shown.

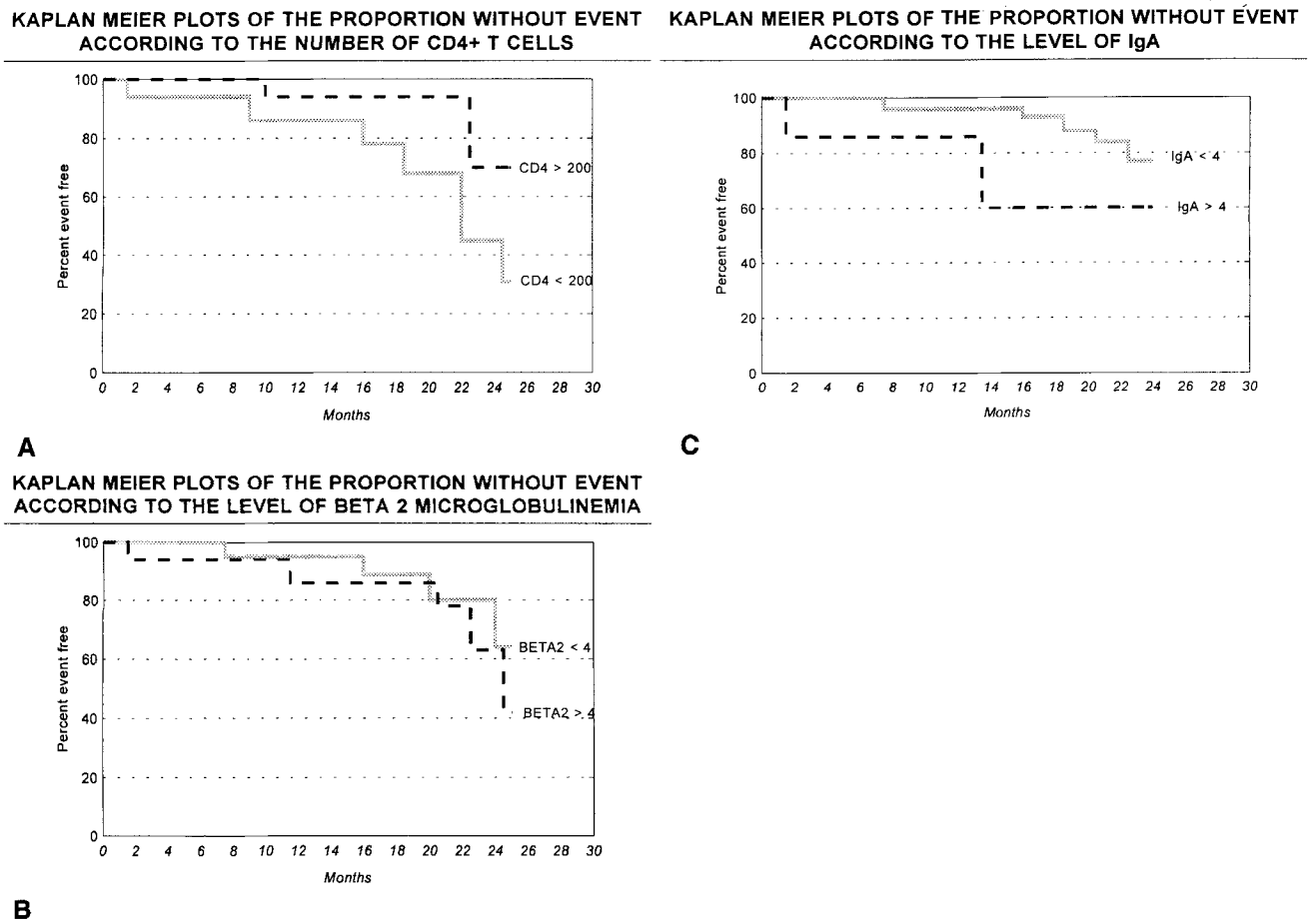

Fig. 1. Progression curves in 45 HIV-1 infected Black patients at early stages of HIV infection, according to the levels of classical surrogate markers for HIV-1 infection at inclusion; **A:** CD4 T cell counts; **B:** serum β2-microglobulin; **C:** serum total IgA.

patients exhibiting a level below 4 g/l, 77.2% (SD = 9.5) after 24 months (Fig. 1C) (Mantel Cox = 2.27, $P = 0.13$).

For each procedure concerning p24 antigenemia determination, the patients were divided according to the presence or absence of p24 antigenemia at the time of enrollment, and the survival curves of p24 antigen-positive group and p24 antigen-negative group were drawn. By method 1, positivity of free p24 antigen at entry was associated with a poor outcome (Fig. 2A): the probability to remain free of event after 24 months was statistically lower in patients having detectable free p24 antigen (n = 3) than in those negative for free p24 antigen (n = 42), respectively 33.3% (SD = 27.2) and 61.2% (SD = 15.5; Mantel Cox = 8.6; $P = 0.003$). Two of the

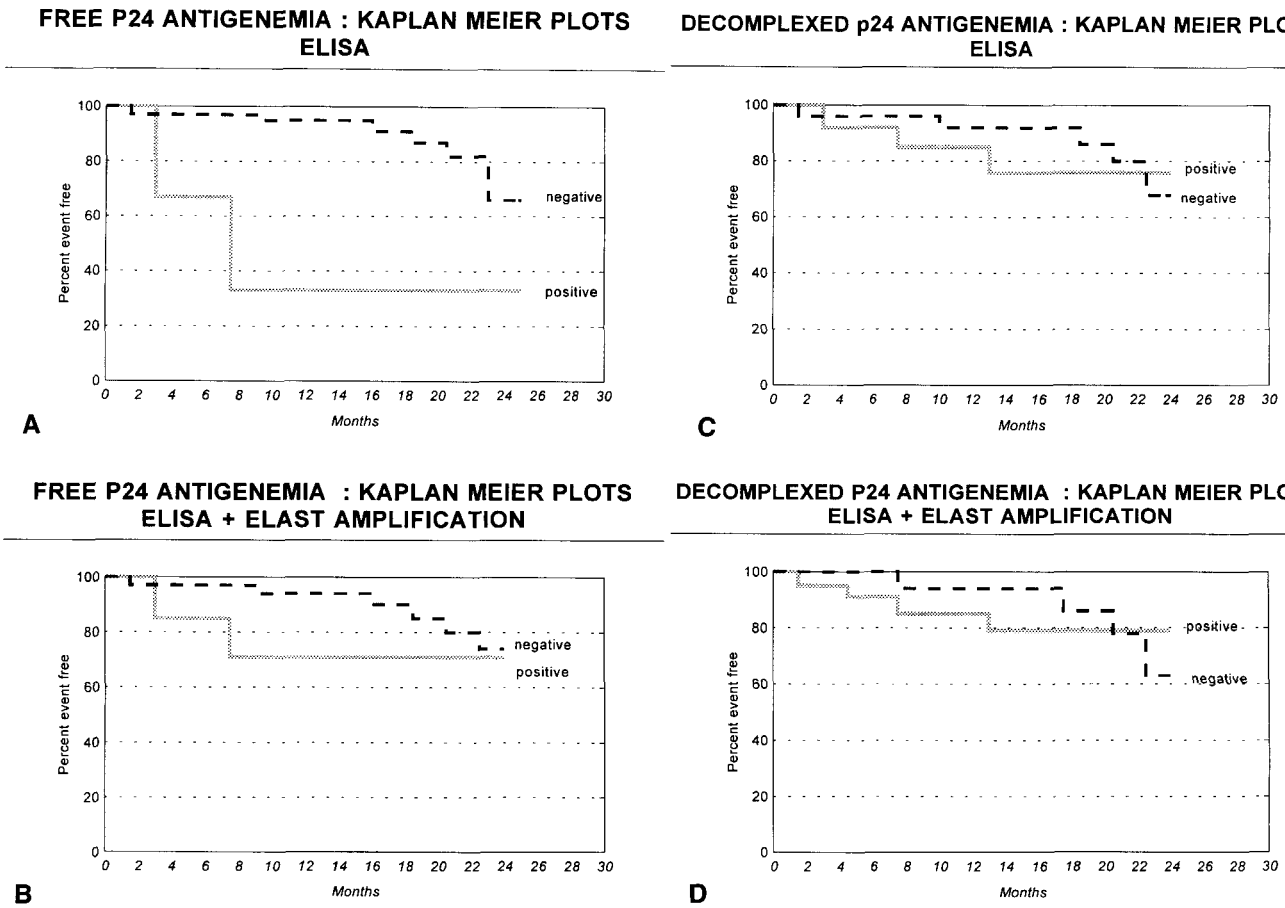

Fig. 2. Proportion of patients without event during follow up according to p24 antigenemia at entry (positive of negative) using different methods of determination. Progression curves in 45 HIV-1 infected Black patients at early stages of HIV infection, according to circulating p24 antigen detection at inclusion; **A:** free p24 antigen measured by immunocapture ELISA (method 1); **B:** free p24 antigen measured by immunocapture ELISA with ELAST™ amplification (method 2); **C:** acid dissociated p24 antigen measured by immunocapture ELISA (method 3); **D:** dissociated p24 antigen measured by immunocapture ELISA with ELAST™ amplification (method 4).

three patients with detectable free p24 antigen, at stage IVC2 and with, respectively, 43 and 153 CD4/mm^3, progressed (events: cerebral toxoplasmosis and oral candidiasis with esophageal candidiasis, respectively), whereas one remained free of event. By method 2, the positivity of p24 antigen at entry had no prognostic value for progression (Fig. 2B): the probability to remain free of event after 24 months was 71.4% (SD = 17.1) in patients positive for p24 antigen (n = 7) and 74.0% (SD = 9.7) in those negative for p24 antigen (n = 38; Mantel Cox = 0.36, P = 0.54). By method 3, similar results were observed (Fig. 2C): the probability to remain free of event within 24 months was 76.6% (SD = 11.9) in patients determined as p24 antigenemia positive (n = 14) and 68.0% (SD = 13.2) in those determined as p24 antigenemia negative (n = 3; Mantel Cox = 0.06, P = 0.80). Finally, the same findings were found by method 4 (Fig. 2D): the probability of remaining free of event within 24 months was 79.0% (SD = 9.2) in patients determined as p24 antigenemia positive (n = 22) and 63.2% (SD = 16.6) in those determined as p24 antigenemia negative (n = 23; Mantel Cox = 0.13, P = 0.71).

The predictive value for a positive p24 antigenemia on the variation of the mean variations of CD4 T-cell count, serum β_2-microglobulin, serum IgA, and of CD4/CD8 ratio was also considered. The positivity of p24 antigenemia at entry did not appear to be significantly predictive of the variations of another surrogate marker after 24 months, whatever method was used to detect p24 antigen (Table IV). However, the decrease in CD4 T-cell counts showed a trend to be more pronounced in patients with positive free p24 antigenemia at entry (−48/mm^3 after 24 months), when compared with those negative for p24 antigenemia at entry (−4/mm^3 after 24 months); however, the threshold of significance was not reached (P = 0.10) because of the limited number of patients included with detectable free p24 antigenemia.

Twenty-nine patients presented a CD4 cell count higher than 200/mm^3 at inclusion. Among those, the prevalence of patients showing a decrease of their CD4 cell count below 200 cells/mm^3 (progressor) appeared independent of the positivity of p24 antigen detection, whatever the method used. For example, 75% of patients with positive free p24 antigenemia and 72% of patients

TABLE IV. Correlation Between p24 Antigenemia Determination and Surrogate Markers at 24 Months*

	CD4	β2 (%)	IgA (%)	CD4/CD8 (%)
Free p24 antigen (ELISA)				
+	−48	50	0	−1
−	−4.5	33	22	−4
Free p24 antigen (ELISA + ELAST)				
+	−16	50	25	+1
−	−5	32	21	−5
Decomplexed p24 antigen (ELISA)				
+	−10	45	33	−6
−	−6	29	17	−3
Decomplexed p24 antigen (ELISA + ELAST)				
+	+1	41	27	−6
−	−16	27	17	−2

*Mean variation of cells and percentage of patients with variation of biological markers at 24 months (i.e., from normal to abnormal levels).

without detectable antigenemia progressed during their follow-up.

The distribution of patients receiving antiretroviral treatment before inclusion or during the follow-up was similar in each group when p24 antigenemia was determined ($\chi^2 = 0.52$, NS). Among the patients receiving antiretroviral therapy, free or dissociated p24 antigen did not have a significant predictive value (data not shown). We did not find any difference of prevalence as of predictive value for free and dissociated p24 antigenemia according to the sex.

DISCUSSION

As surrogate markers assume greater importance in disease staging and evaluation of antiretroviral therapy, it is important to note that the prevalence of free p24 antigenemia may be much lower in HIV-1-infected Black patients than in Caucasians [Baillou et al., 1987; Bélec et al., 1993]. The potential interest of the acid pretreatment procedure to increase the sensitivity of p24 antigenemia detection in HIV-1-infected Caucasian [Nishanian et al., 1990] as well as in Black people [Bélec et al., 1993; Brown et al., 1995] has been underlined previously. Acid treatment of the sera provides for dissociation of the circulating immune complexes, as well as denaturation of the specific anti-p24 antibodies, while p24 antigen immunoreactivity is preserved [Nishanian et al., 1990]. In the present study, the acid dissocation procedure significantly increased the rate of detection of circulating p24 antigen. Furthermore, the acid dissociation of the serum immune complexes associated with the ELAST™ amplification system appeared more sensitive than acid dissociation alone for detecting circulating free p24 antigen, as well as circulating p24 antigen hidden in immune complexes. Such methods appear useful, relatively simple, and inexpensive to increase the efficacy of p24 antigen detection in HIV-1-infected Black people. One possible application of this method in virological diagnosis could be the detection of circulating p24 antigen in children born to HIV-1-infected mothers [Miles et al., 1993], especially in developing countries where sophisticated facilities for HIV diagnosis are lacking.

We evaluated further the prognostic value for progression of classical surrogate markers, and of free or dissociated p24 antigenemia, in a series of 45 HIV-1-infected Black patients at non-AIDS stages prospectively included and followed up during a mean of 30 months. Among indirect surrogate markers, only the CD4 T-cell count was predictive for disease progression or death. Patients with detectable free p24 antigen at inclusion showed a higher progression of HIV infection at 24 months than those with undetectable free p24 antigen: two of them progressed whereas one remained free of event. Although the number of free p24 antigen-positive patients was rather limited in our series, this observation suggests a seemingly good prognostic value at 24 months of free p24 antigenemia in HIV-1-infected Black people, probably because of the very high viral burden when free p24 becomes detectable. By contrast, the positivity of p24 antigenemia, as determined by amplification procedure or after acid pretreatment of the sera, did not appear to be predictive of disease progression at 30 months in HIV-1-infected Black patients at non-AIDS stages.

Our observations contrast also with two previous studies focused mainly on Caucasian patients, demonstrating a relatively good predictive value of dissociated p24 antigen in naive patients [Morand-Joubert et al., 1994] as well as in AZT-treated patients [Bollinger et al., 1992]. However, the predictive value for progression of dissociated p24 antigenemia remains controversial [Morand-Joubert et al., 1995]. Furthermore, dissociated p24 antigen appears clearly less predictive of treatment efficacy than HIV/RNA viral load, which is more sensitive and demonstrates a more dynamic range than p24 antigenemia [Kappes et al., 1995]. Finally, it is conceivable that the more extensive binding of antibodies to p24 antigen in Blacks than in Caucasian patients could have resulted in partial immune complex association in sera from our patients.

In conclusion, free p24 antigenemia may be predictive of progression at 24 months in HIV-1-infected Black patients, but this marker lacks a great deal of sensitivity. By contrast, dissociated p24 antigenemia does not appear to have predictive value for progression in HIV-1-infected Black patients at early stages of HIV infection; therefore, it cannot be recommended as a valuable surrogate marker in such patients. Finally, as determination of circulating viral load becomes more easily assessed by use of methods in molecular biology, an increase in p24 antigenemia sensitivity does not appear to be a major case in the management of HIV-1-infected individuals, especially in Black people.

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